Ultrasonic Activation of PVDF Film Enables Piezopotential-Induced Hydroxyapatite Mineralization

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Materials. Poly(vinylidene fluoride) (PVDF), Alizarin Red S, Sodium chloride, sodium bicarbonate, potassium chloride, dipotassium hydrogen phosphate trihydrate, magnesium chloride hexahydrate, calcium chloride, sodium sulfate, and Dimethylformamide (DMF) ware purchased from Merck and used without further purification.

Instrumentation. Transmission electron microscopic (TEM) images were obtained by using JEOL, JEM 2100 F microscope equipped with 200 kV electron source. The surface chemical composition of the film was investigated by using Omicron: serial number 0571 X-ray photoelectron spectrometer (XPS) equipped with an Al K_α X-ray source and hemispherical analyzer. The field emission electron microscopy (FESEM) images of the film were recorded by employing Supra 40 Carl Zeiss Pvt. Ltd. instrument. To investigate piezopotential generation under ultrasonic cavitation, a three-dimensional PVDF membrane model (1 × 1 × 0.1 cm) was designed in COMSOL Multiphysics 6.1. A uniform axial compressive pressure of 10^8 Pa (representing the pressure generated during ultrasonic bubble collapse) was applied to the top surface, while the bottom surface was fixed. The resulting volumetric strain and piezopotential distribution generated across PVDF membrane was subsequently mapped in COMSOL Multiphysics 6.1. Piezoelectric force microscopy (PFM) was performed at room temperature with an Asylum Research MFP-3D atomic force microscope (AFM) with a Ti/Ir (5/20) coated cantilever (ASYELEC-01, nominal spring constant of 2.0 N/m, and resonance frequency of 81 kHz). The piezoelectric "butterfly" loop ware recorded under DC voltage from −7 to +7 V. The piezo current and voltage response were recorded by employing Keithley DMM6500 mm. The FTIR measurement was carried out by using PerkinElmer Spectrum 100 FTIR spectrometer. The florescence microscopic images and the differential interference contrast (DIC) were done by Olympus IX81 microscope using Image-Pro Plus version 7.0 software. The X-ray Diffraction (XRD) measurement was done by Bruker D8 advance powder diffractometer equipped with Cu Kα X-ray radiation (λ = 1.5406 Å) source. ICP analysis was performed using an Optima 2100 DV (PerkinElmer) inductively coupled plasma optical emission spectroscope (ICP-OES) against standard solutions.

Synthesis of the PVDF film. The PVDF film was prepared by previously reported method. Briefly, 500 mg PVDF were dissolved into 10 ml DMF at 60^{o}C under stirring condition at 600 rpm for 2h. Subsequently, the mixture was transferred into a Petri dish and dried in a hot air oven for 10 hours. After drying, the films were carefully peeled off from the Petri dish.

Bio-mineralisation technique. The film was cut into 2×2 cm^2 section. Then the square section film was merged in the 10 mL 10 X SBF solution. Then the solution was treated with ultrasound (1.0 w/cm^2 power, 1 MHz frequency, 50% duty cycle) for 2 h. After 2h of ultrasonic exposure the film was carefully retrieved from the solution and allowed to dry under ambient conditions for further experimentation.

Alizarin red S assays. Mineralized PVDF film was cut into 0.5×0.5 cm^2 square. Then, the square film was stained with 1 mg/mL Alizarin Red S in aqueous buffer for 20 min under ambient condition. Then the stained film rinsed gently with deionized water. After that, the film was merged in PBS solution for 2 min. The imaging of the film was done by using Olympus IX81 microscope.

Piezopotential generation under ultrasound. The as-prepared films were first cut into 1 × 1 cm dimensions and subjected to recording the piezo-electromechanical performance. For this, silver paste (supplied by Acheson Colloiden B.V. The Netherlands) was painted on the surface of the film maintaining ~5 mm between the two electrodes. Fine copper wires were attached to establish electrical connections between the samples and Keithley DMM6500 mm. To study ultrasound assisted piezopotential generation, the films were laminated with polyethylene and placed in a 50 mL water-filled beaker. Then, an ultrasonic transducer was placed in the beaker to the beaker to provide acoustic energy to the films. The piezopotential response was measured using Keithley DMM6500 mm.

Current-voltage response measurement. Initially commercial malleable aluminium (Al) foils were thinned using 1800 grids and paper and then cleaned by ultrasonication in water and ethanol for 15 min. Then mineralized membrane was attached on 1 × 1 cm^2 of Al foil with another thinned flexible Al foil was attached onto the upper surface so that the foil that acted as the upper electrode. When required, a thin layer of silver (Ag) paint (supplied by Acheson Colloiden B.V. The Netherlands) is also used beneath the Al foil to ensure proper adhesion of the Al foil to the sample surface. Fine copper wires were attached to establish the electrical connections between the samples and Keithley DMM6500 millimeter. The mechanical stress on the piezoelectric samples was applied using a custom-built setup controlled by the LabVIEW program. This setup utilized uniaxial compressive force to apply stress on the samples. The samples were positioned between two plates, and a controlled force was applied to apply the stress. The magnitude of the applied mechanical stress was precisely regulated through the LabVIEW program. The forces exerted on the device were found to be 3 N. The piezo current and voltage of the fabricated samples was measured by using a Keithley DMM6500 millimeter. Additionally Kapton tapes were used to interface the device for monitoring human movements.

Calculation of different phases of PVDF. The electroactive polar phases of the mineralised PVDF film were calculated by FTIR by using following equation:

$$F(polar) = \frac{A_\beta}{\left(\frac{K_\beta}{K_\alpha}\right)A_\alpha + A_\beta} \text{……..eqS1}$$

The K_α and K_β are the constant with values 7.7 × 10^4 and 6.1 × 10^4 cm^2/mol, respectively, the A_β and A_α are represented the absorbance values at 841 and 765 cm^{-1} respectively.

We have further calculated the contribution of the β and γ phase of the mineralized PVDF film. The β and γ phase calculated by the deconvolation of the peak at ~841 cm^{-1}. After deconvulation the β and γ phase of the mineralized film was calculated by employing following equation:

$$F(\beta) = F(polar) \times \frac{A_\beta}{A_\beta + A_\gamma} \text{……….eqS2}$$

$$F(\gamma) = F(polar) \times \frac{A_\gamma}{A_\beta + A_\gamma} \text{……….eqS3}$$

The A_β and A_γ correspond to the integrated areas of the β and γ peaks respectively. The detailed calculated phases of the films are given in the table S2.

Protocol for the preparation of the 10X SBF solution. The detailed recipe for preparing 1 L of 10X SBF solution is provided in Table S1. The salts were added sequentially to 1 L of water in the order listed in Table S1.

Table S1. Recipe for the preparation of the 10X SBF solution

Salt	Weight (g/L)
NaCl	58.443
KCl	0.373
$CaCl_2$	2.775
$MgCl_2.6H_2O$	1.017
NaH_2PO_4	1.200
$NaHCO_3$	0.840

Table S2. Different phases of the PVDF membrane. Control 1 represents as prepared PVDF films, control 2 represents PVDF films immersed in SBF solution, control 3 represents PVDF films exposed to ultrasound in aqueous solution.

Phase	Control 1	Control 2	Control 3	Mineralised PVDF film
F(polar)	63%	56%	63%	71%
F(β)	33%	29%	38%	53%
F(γ)	30%	26%	25%	18%

Table S3. Summary of reported piezoelectric scaffolds for mineralisation under incubation/mechanical loading/ultrasound.

Composition	Piezoelectric constant (d33)	Mineralized Technique	Time	Reference
poly[(*R*)3-hydroxybutyrate] (PHB) and poly[3-hydroxybutyrate-*co*-3-hydroxyvalerate] (PHBV) 3D scaffold	**PHB**: 3.0 ± 0.5 pC/N **PHBV:** 0.7 ± 0.5 pC/N	Incubation	7 days	35
PVDF	≈ 2 pC/N	Incubation	4 days	13
50% $BaTiO_3$-50% HAp composite	≈ 9 pC/N	Incubation	7 Days	36
poly(-caprolactone)-poly(ethylene glycol)–poly(-caprolactone)	-	Incubation	21 Days	37
$BaTiO_3$ filler in dental resin composites	1.35 pC/N	Incubation	7 days	38
TiO_2–$BaTiO_3$	0.71 pC/N	Incubation	7 days	39
calcium phosphate cement -20% $BaTiO_3$	-	Incubation	7 days	40
calcium phosphate silicate (CPS)-doped PVDF scaffold	~ 3 pC/N	Incubation	28 days	41
graphene (G)/barium titanate (BaTiO3)/Magnesium phosphate cement	~6.31 pC/N	Incubation	14 days	42
PVDF	~15 pC/N	ultrasound	2h	this work

Table S4. Comparison of piezoelectric/energy harvesting capability of mineralised PVDF membranes with reported works.

Composition	Fabrication technique	Electrical voltage and current output	Piezoelectric coefficient (d33)	Applications	Reference
9 wt% HAp nanowires +PVDF	Solvent evaporation method	Voltage ~2.6 V; current 10 µA	≈ 58 pm/V	ultrasound-assisted electroporation	24
8.2 vol% HAp + PVDF nanorod	Insitu preparetion of HAp followed by solvent evaporation	Voltage ~47 V ; current ~1.8 µA	≈ 56.6 pC/N	electron generator by absorbing light energy	43
25 wt% HAp/PVDF-hexafluoropropylene nanofiber film	curing and heating followed by electro-supnning	Voltage ~1.1 V	30.2442 pm/V	piezoelectric nanogenerator	44
HAp-PLLA film	electrospinning	-	4.58 ± 0.08 pC/N	Induced cell growth and accelerates regenerative processes	45
9 wt% HAp -PVDF film	high energy ball milling (HEBM)	-	58 pm/V	bone regeneration	46
HAp-PVDF film	Mineralization	Voltage ~1.9 V ; current ~8 µA	42 pm/V	**Human motion sensors**	this work

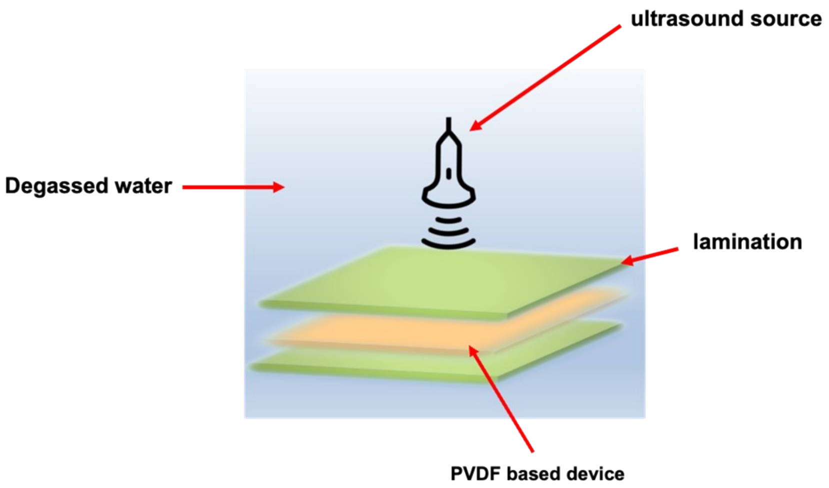

Figure S1. Schematics of experimental setup for piezopotential measurements.

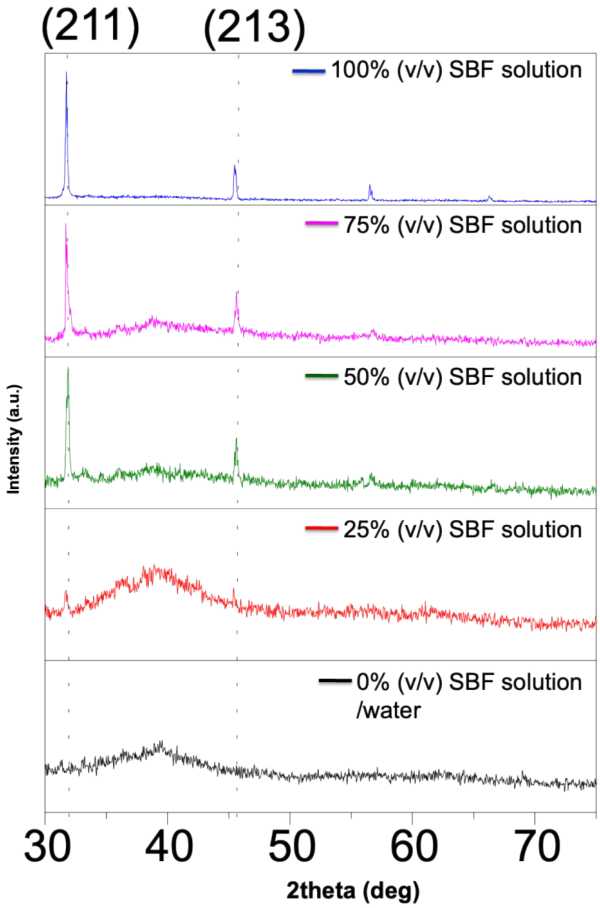

Figure S2. XRD of hydroxyapatite deposition on PVDF under different concentration of 10X SBF solution. Where 0% means pure water, 25% denotes 25% (v/v) dilution of SBF in water; 50% denotes 50% (v/v) dilution of SBF in water; 75% denotes 75% (v/v) dilution of SBF in water and 100% pure SBF solution.

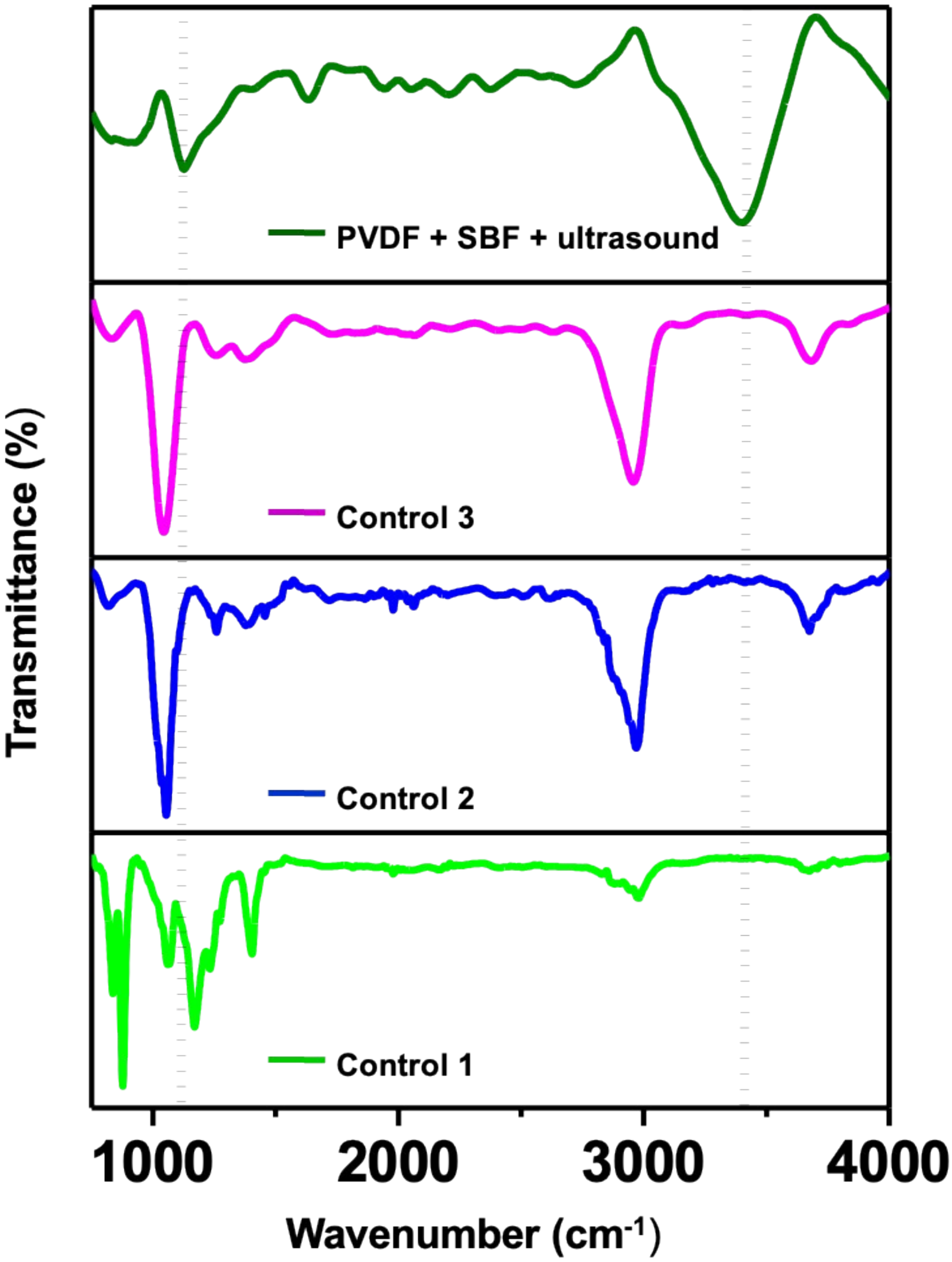

Figure S3. FTIR spectra of the PVDF membranes immersed in SBF solution exposed to ultrasound (2h). **Control 1** represents as prepared PVDF films, **control 2** represents PVDF films immersed in SBF solution, **control 3** represents PVDF films exposed to ultrasound in aqueous solution.

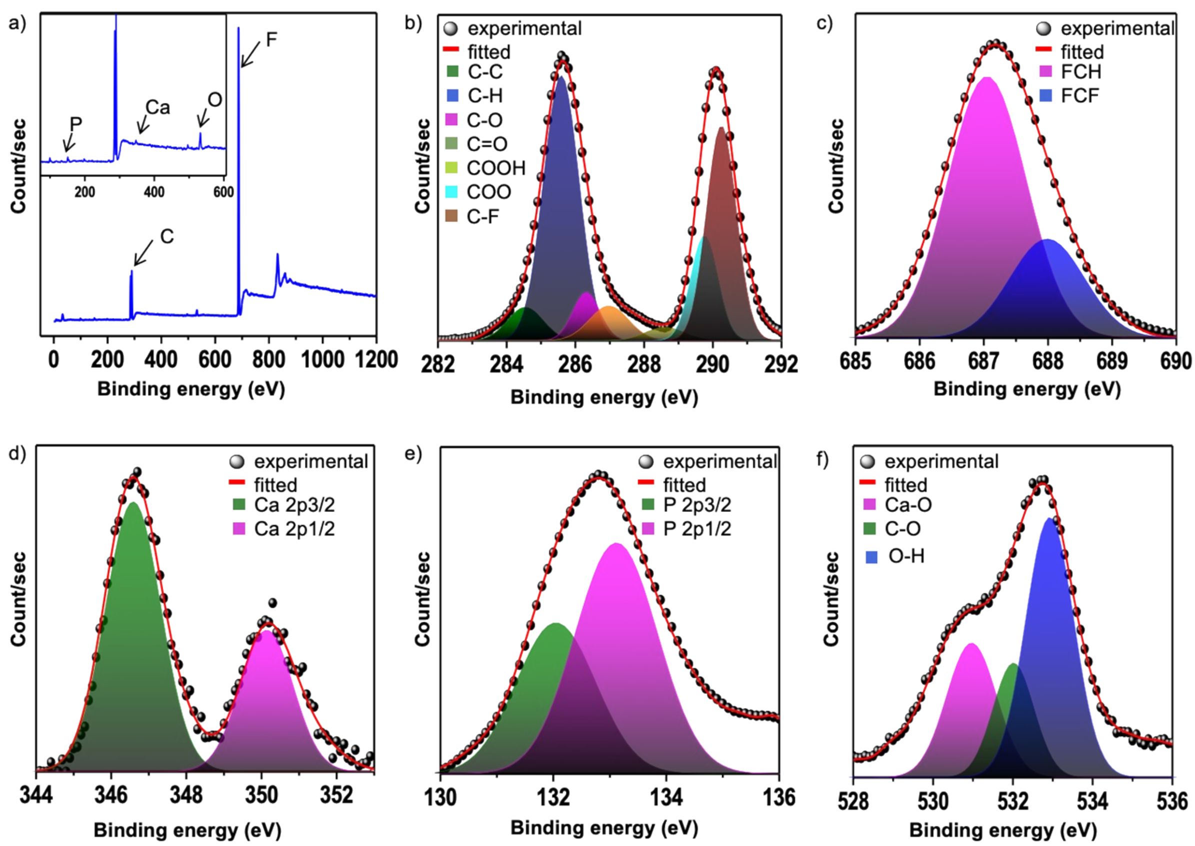

Figure S4. (a) Survey scan X-ray photoelectron spectroscopy (XPS) of the hydroxyapatite deposited PVDF films in inset zoomed survey scan (95-600 ev), (b-f) Deconvoluted XPS spectrum of hydroxyapatite deposited PVDF films: (b) C 1s, (c) F 1s, (d) Ca 2p, and (e) P 2p, (f) O 1s.

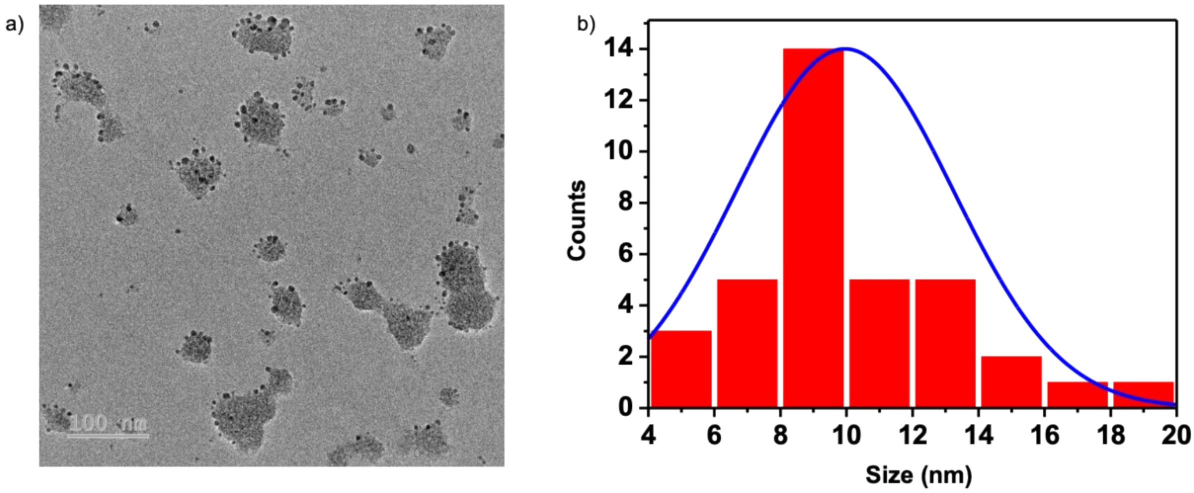

Figure S5. TEM images of the hydroxyapatite deposited PVDF film (a) and its corresponding histogram analysis (b).The mineralized PVDF membrane was dissolved in toluene under sonication, and the dilute solution was used for grid preparation.

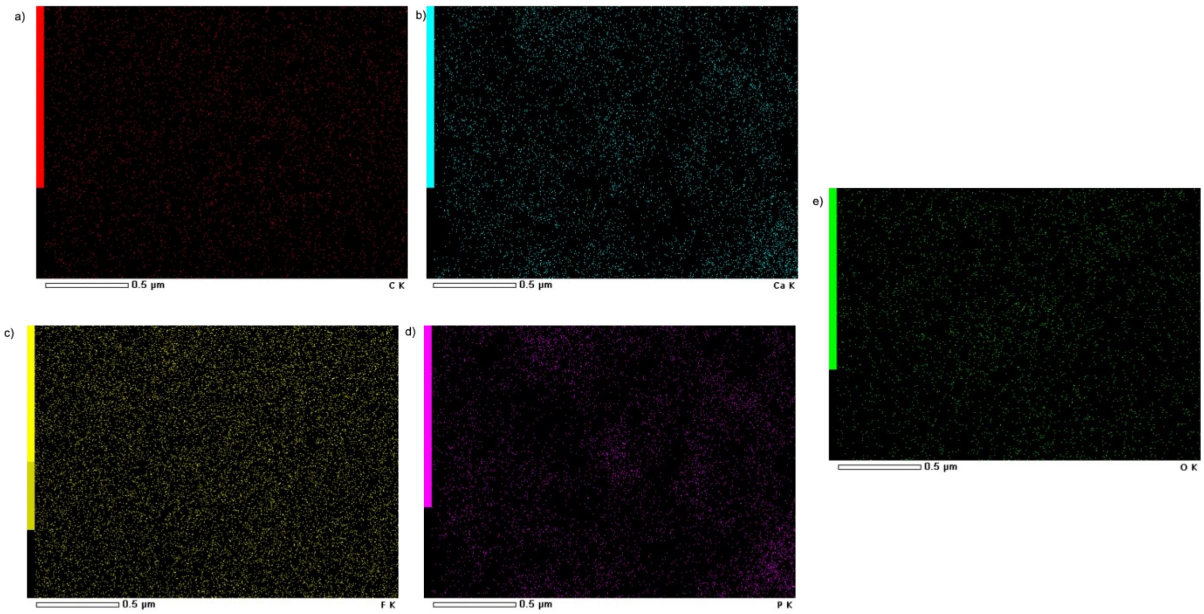

Figure S6. Elemental mapping of the hydroxyapatite deposited PVDF film. (a) C; (b) Ca; (c) F; (d) P ; (e) O.

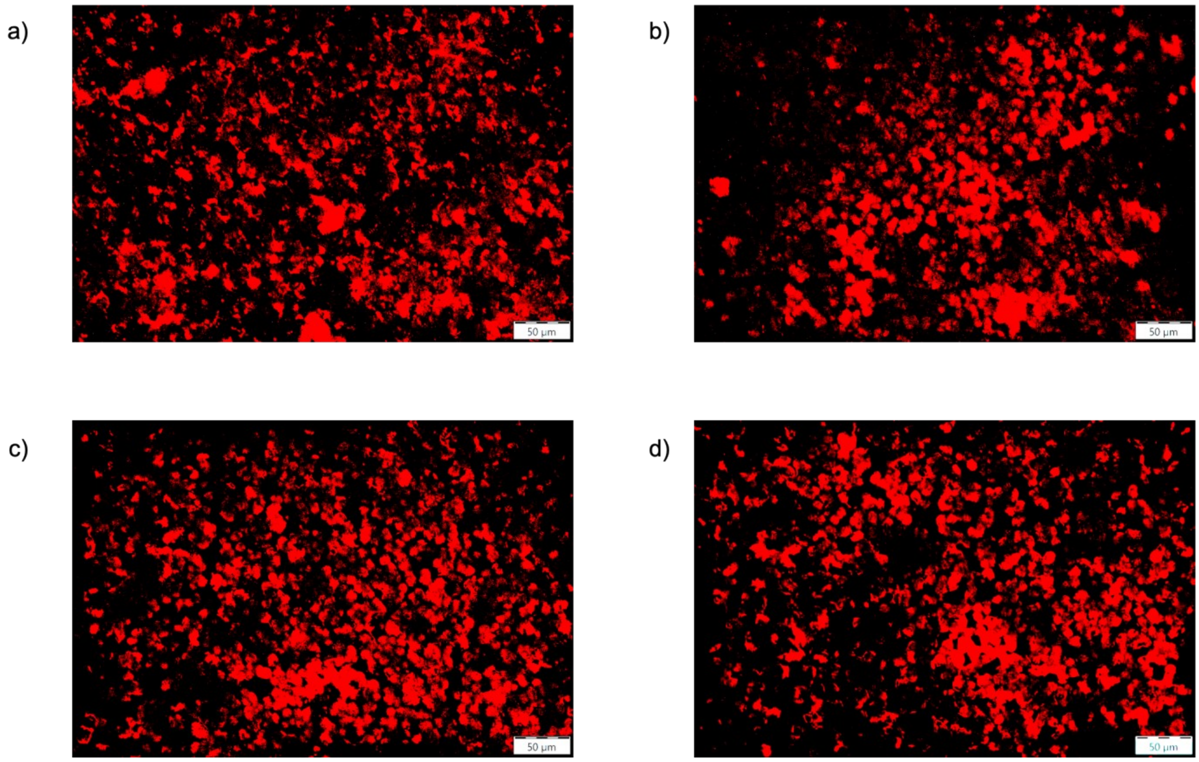

Figure S7. Alizarin S staining of hydroxyapatite formed on PVDF films under ultrasonic exposure in SBF solution after different mineralization cycles: one (a), two (b), three (c), and four (d). Scale bar is 0.5 µm.

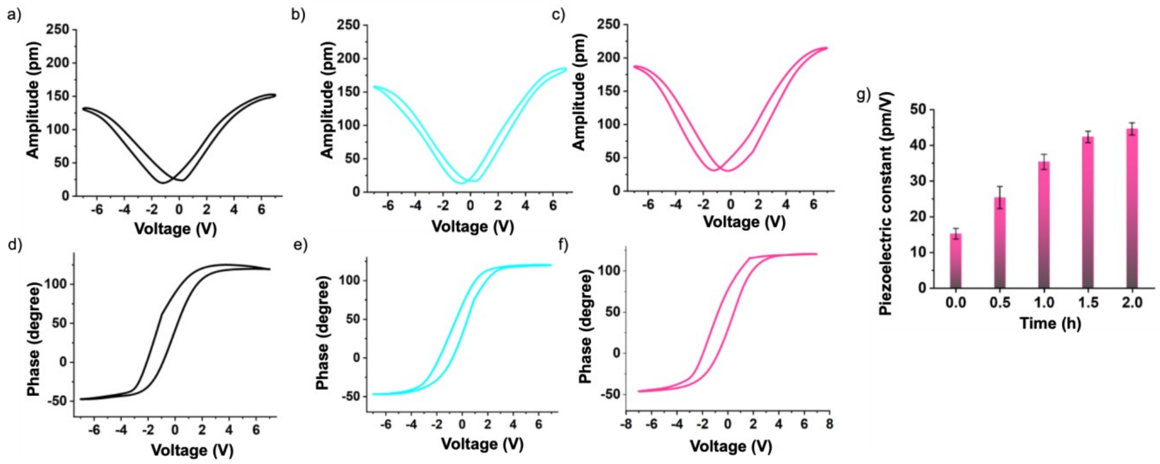

Figure S8. Piezoresponse force microscopy (PFM)-based amplitude vs. voltage butterfly curve (a-c) and phase vs. voltage hysteresis curve (d-f) of the PVDF film with varied ultrasound exposure time. a,d) 0.5h; b,e) 1h; c,f) 2h. g) Piezoelectric constant of the film under differ ultrasound treatment time.

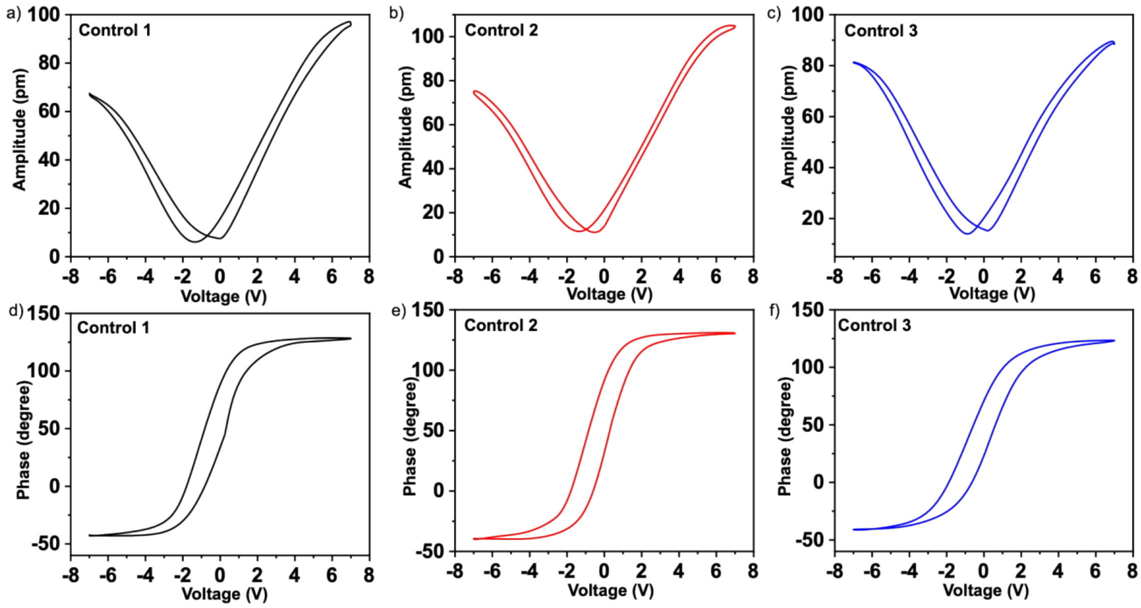

Figure S9. PFM analysis of the PVDF film. **Control 1** represents as prepared PVDF films (a,d), **control 2**represents PVDF films immersed in SBF solution (b,e), **control 3** represents PVDF films exposed to ultrasound in aqueous solution.(c,f)

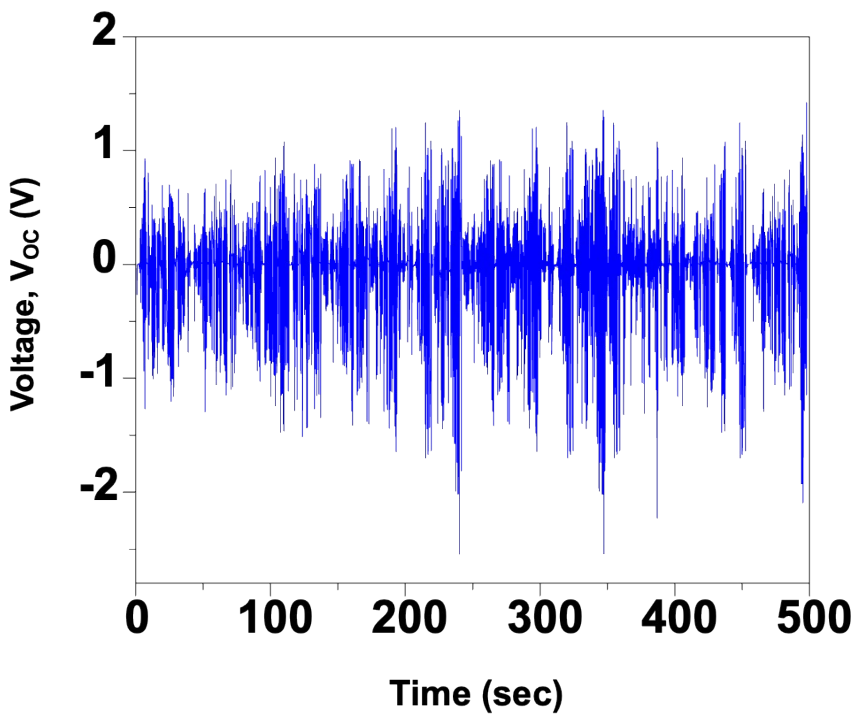

Figure S10. Long term electrical output of mineralized PVDF membrane.

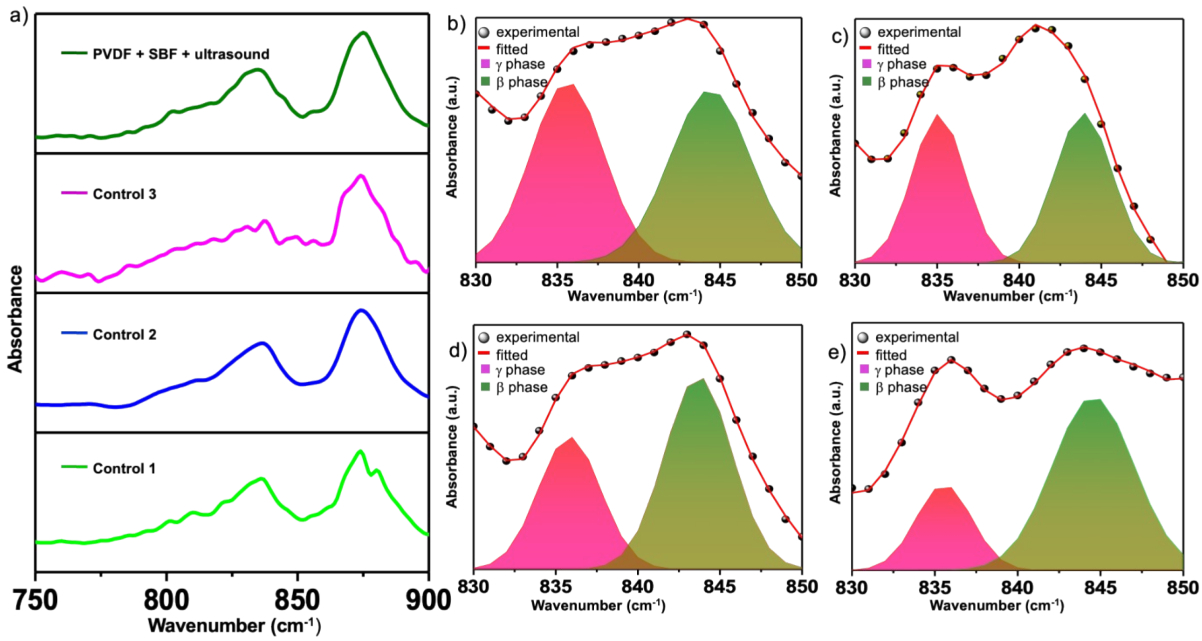

Figure S11. FTIR spectra of PVDF film (a). Deconvoluted XPS spectrum of the PVDF films showing b and g phase (b) Control 1, (c) Control 2, (d) Control 3, (e) SBF solution exposed to ultrasound (2h). **Control 1** represents as prepared PVDF films, **control 2** represents PVDF films immersed in SBF solution, **control 3** represents PVDF films exposed to ultrasound in aqueous solution.